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Transformation of the chlorarachniophyte *Amorphochlora amoebiformis* by electroporation V.2

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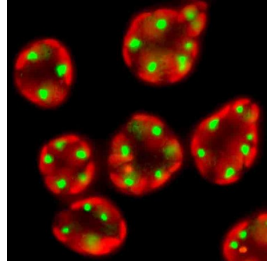
Kodai Fukuba¹, Liz Cooney², Nick Irwin³, Patrick J. Keeling³, Yoshihisa Hirakawa¹

¹University of Tsukuba; ²University of British Columbia (CA); ³University of British Columbia



Yoshihisa Hirakawa

University of Tsukuba



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Protocol status: Working

We use this protocol and it's working

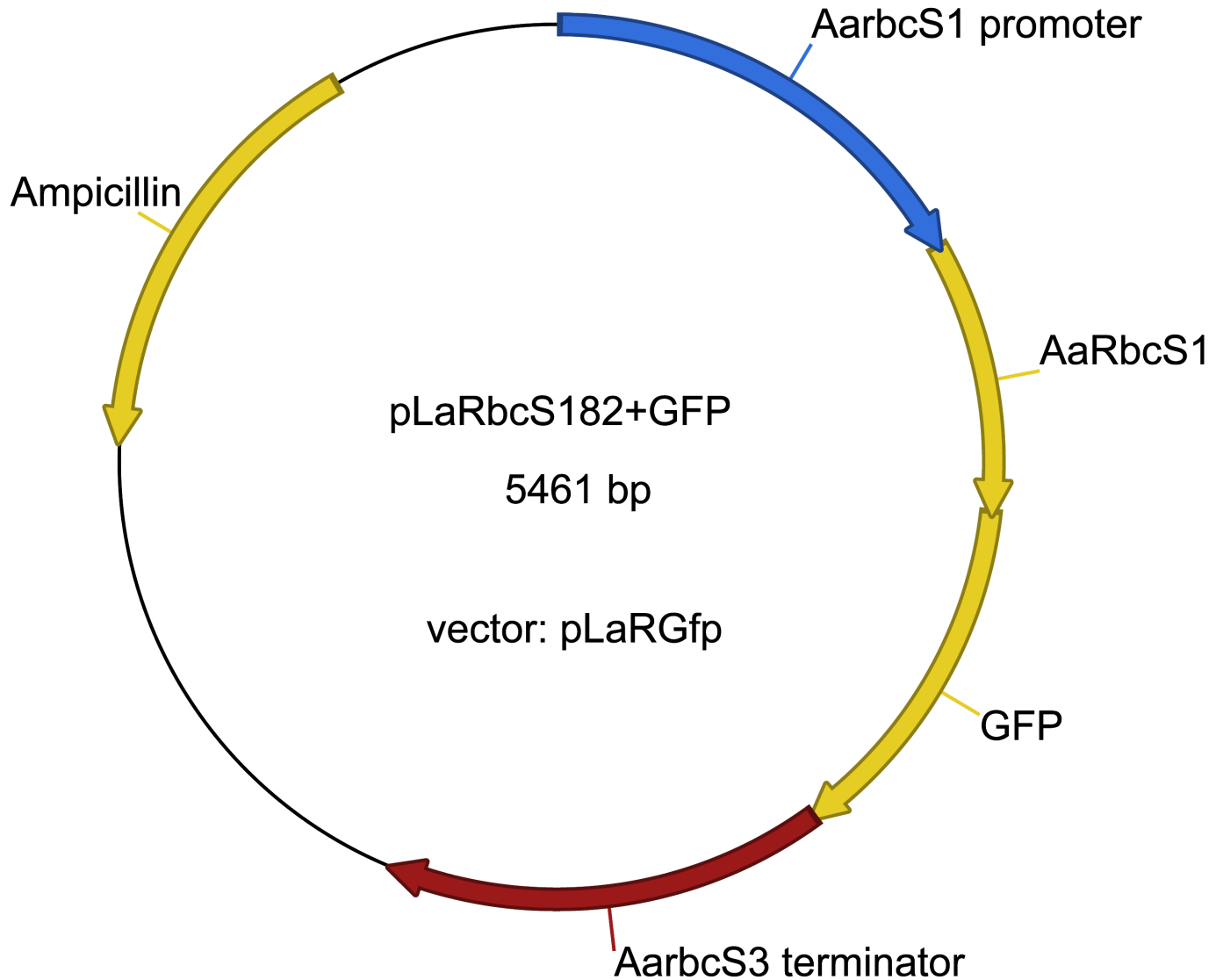
Created: June 13, 2019

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Materials

Plasmid information



>pLaRbcS182+GFP

GAAC TCGAGCAGCTGCACACATTAACATTCATGTGGATAATAAATATAATATATATATATATATATTATATATATATATATATACA

Hirakawa Y. Ishida K. (2010) Internal plastid-targeting signal found in a RubisCO small subunit protein of a chlorarachniophyte alga. *The Plant Journal*. 64: 402-410.

Plasmid preparation

- 1 Propagate plasmid DNA (pLaRGfp or its derivatives, such as pLaR182+GFP) in the *Escherichia coli* strain DH5 α .

CITATION

Hirakawa, Y., Ishida, K. (2010). Internal plastid-targeting signal found in a RubisCO small subunit protein of a chlorarachniophyte alga. *The Plant Journal*.

LINK

[10.1111/j.1365-313X.2010.04334.x](https://doi.org/10.1111/j.1365-313X.2010.04334.x)

CITATION

Hirakawa, Y., Kofuji, R., Ishida, K. (2008). Transient transformation of a chlorarachniophyte alga, *Lotharella amoebiformis* (chlorarachniophyceae), with uidA and egfp reporter genes. *Journal of Phycology*.

LINK

[10.1111/j.1529-8817.2008.00513.x](https://doi.org/10.1111/j.1529-8817.2008.00513.x)

- 2 Purify plasmid DNA from 200 mL culture of *E. coli* by a Qiagen Plasmid Maxi Kit (Qiagen).
- 3 Adjust plasmid DNA concentration to 3-5 $\mu\text{g}/\mu\text{L}$ with distilled water.

Cell culture

- 4 Culture *Amorphochlora amoebiformis* (CCMP2058) cells in 500 mL Erlenmeyer flasks containing 200 mL ESM medium at 20°C under white illumination (50-80 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on a 14:10 hours light:dark cycle for a week.



- 5 After decanting medium, resuspend cells adhered to the bottom of flasks by gentle pipetting with 2 to 3 mL ESM medium (use a glass pipette). Approximately 4×10^7 cells can be obtained from a flask.

Electroporation

- 6 Harvest a total of 5×10^6 cells by centrifugation at 2,000 g for 5 sec (use a mini centrifuge).
- 7 Resuspend cell pellet in 100 μ L of Gene Pulser electroporation buffer (Bio-Rad) with 10 μ L of plasmid DNA at the room temperature.
- 8 Transfer cell solution into electroporation cuvette with 0.2 cm gap (Bio-Rad).
- 9 Electroporate cells with a 25 ms square wave pulse at 120 V using Gene Pulser Xcell Electroporation System.
- 10 Add 0.9 mL fresh ESM medium to cuvette immediately after electroporation.
- 11 Transfer cells to glass bottom well plate/petri dish, and add an appropriate volume of ESM medium.
- 12 Incubate cells for 24 hours before observation of GFP fluorescence.

Citations

Step 1

Hirakawa, Y., Kofuji, R., Ishida, K.. Transient transformation of a chlorarachniophyte alga, *Lotharella amoebiformis* (chlorarachniophyceae), with uidA and egfp reporter genes
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